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(54) Title: 2,6-DIDEOXY-2,3-DIDEHYDRO-6-THIO DERIVATIVES OF α-D-NEURAMINIC ACID

(57) Abstract

The invention provides 2,6-dideoxy-2,3-didehydro-6-thio derivatives of α-D-neuraminic acid. Methods for preparation of the compounds of the invention, pharmaceutical formulations comprising the compounds, and use of the compounds as anti-viral agents are also disclosed and claimed.

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2,6-DIDEOXY-2,3-DIDEHYDRO-6-THIO DERIVATIVES OF α-D-NEURAMINIC ACID

This invention relates to a new class of chemical compounds and to their use in medicine. In particular the invention concerns new 2,6-dideoxy-2,3-didehydro-6-thio derivatives of α-D-neuraminic acid, methods for their preparation, pharmaceutical formulations thereof and their use as anti-viral agents.

Enzymes with the ability to cleave *N*-acetyl neuraminic acid (NANA), also known as sialic acid, from other sugars are present in many microorganisms. These include bacteria such as *Vibrio cholerae*, *Clostridium perfringens*, *Streptococcus pneumoniae*, and *Arthrobacter sialophilus*, and viruses such as influenza virus, parainfluenza virus, mumps virus, Newcastle disease virus, fowl plague virus, and Sendai virus. Most of these viruses are of the orthomyxovirus or paramyxovirus groups, and carry a neuraminidase activity on the surface of the virus particles. It is noted that neuraminidase is also known as sialidase.

Many of the neuraminidase-possessing organisms are major pathogens of man and/or animals, and some, such as influenza virus, Newcastle disease virus, and fowl plague virus, cause diseases of enormous economic importance.

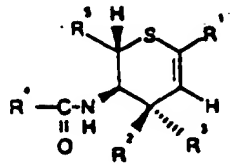
It has long been thought that inhibitors of neuraminidase activity might prevent infection by neuraminidase-bearing viruses. Most of the known neuraminidase inhibitors are analogues of neuraminic acid, such as 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid (DANA) and its derivatives. See, e.g., Meindl et al., Virology, 1974 58 457-463. The most active of these is 2-deoxy-2,3-dehydro-*N*-trifluoroacetyl-neuraminic acid (FANA), which inhibits multi-cycle replication of influenza and parainfluenza viruses *in vitro*. See Palese et al, Virology 1974 59 490-498.

Our earlier international application Publication No. WO 91/16320 describes a number of analogues of DANA. The entire disclosure of this specification is herein incorporated by reference. The compounds disclosed in WO 91/16320 are active both *in vitro* and *in vivo* against viral neuraminidase, and are useful in the treatment of influenza.

We have now found further compounds which fall generally within the scope of the compounds described and claimed in WO 91/16320, but which are not specifically disclosed therein. These compounds have particularly advantageous properties.

Summary of the Invention

The invention therefore provides in a first aspect compounds of formula (I)



(I)

wherein R^1 is COOH, $P(O)(OH)_2$, NO_2, SOOH, SO_3H, tetrazol, CH_2CHO, CHO, or $CH(CHO)_2$;

one of R^2 and R^3 is OH, $(alk)_xNR^6R^7$, CN or N_3, and the other is hydrogen, where alk is unsubstituted or substituted methylene, and x is 0 or 1, with the proviso that when R^2 or R^3 is OH, R^1 cannot be COOH;

R^4 is methyl, in which one or more hydrogens is optionally replaced by a substituted or unsubstituted C_{1-4}alkyl or aryl group, or by a halogen;

R^5 is $CHOR^9CHOR^9CH_2OR^9$;

R^6 is hydrogen, C_{1-6}alkyl (e.g. methyl, ethyl), allyl, aryl (e.g. phenyl), aralkyl (e.g. phenC_{1-3}alkyl such as benzyl), amidine, NR^7R^8, or an unsaturated or saturated ring containing one or more heteroatoms such as nitrogen,

oxygen or sulphur;

R^7 is hydrogen, C_{1-6}alkyl (e.g. methyl, ethyl), r allyl; or NR^6R^7 forms an optionally substituted 5 or 6 membered ring optionally containing one or more additional heteroatoms such as nitrogen, oxygen or sulphur, or R^6 and R^7 may be the same; and

R^8 is hydrogen or C_{1-6}alkyl; and

each R^9 is the same or different, and is hydrogen; an acyl group having 1 to 4 carbon atoms; a linear or cyclic alkyl group having 1 to 6 carbon atoms, or a halogen-substituted analogue thereof; an allyl group or an unsubstituted aryl group; or an aryl substituted by a halogen, an OH group, an NO_2 group, an NH_2 group or a COOH group;

and pharmaceutically acceptable salts of the compounds of formula (I) and pharmaceutically acceptable derivatives thereof.

In the compounds of formula (I) the substituents (for example the group R^6 in the substituent R^1), may themselves bear substituents conventionally associated in the art of pharmaceutical chemistry with such substituents.

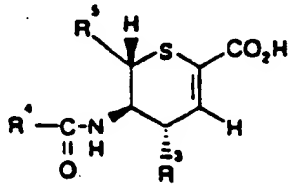
Preferably R^1 is COOH, and R^2 is H.

Preferably R^3 is NR^6R^7, more preferably NH_2 or guanidino.

Preferably R^4 is methyl or halogen-substituted methyl, e.g. FCH_2, F_2CH, CF_3. More preferably R^4 is methyl.

Preferably R^5 is hydrogen or acetyl.

A preferred class of compounds of formula (I) are those of formula (Ia)

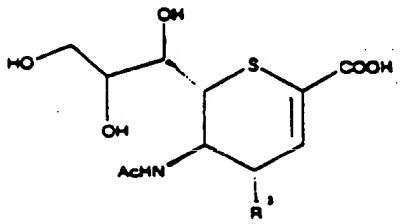


(Ia)

wherein R^3, R^4 and R^5 and X are as defined for formula (I) and pharmaceutically acceptable salts and derivatives thereof.

C_{1-4}alkyl as used herein includes both straight chain (e.g. methyl, ethyl) and branched chain (e.g. isopropyl, t-butyl) alkyl groups.

In a particularly preferred embodiment the invention provides a compound of formula (II),



(II)

wherein R^3 is NH_2 or $NHC(NH)(NH_2)$, and physiologically acceptable derivatives and solvates, such as hydrates, thereof.

Representative compounds within this embodiment include 5-acetamido-4-azido-2,3,4,5,6-pentadeoxy-6-thio-D-glycero-D-galacto-non-2-endopyranosonic acid, 5-acetamido-4-amino-2,3,4,5,6-pentadeoxy-6-thio-D-glycero-D-galacto-non-2-endopyranosonic acid, and 5-acetamido-4-guanidino-2,3,4,5,6-pentadeoxy-6-thio-D-glycero-D-galacto-non-2-endopyranosonic acid, and physiologically acceptable salts and esters thereof.

The person skilled in the art will understand that substituents at the 4-position can be interconverted as described in WO 91/16320.

By pharmaceutically acceptable derivative is meant any pharmaceutically acceptable salt, ester or salt of such ester of the compounds of formula (I) or any other compound which upon administration to the recipient is capable of providing, directly or indirectly, a compound of formula (I) or an anti-virally active metabolite or residue thereof.

It will be appreciated by th se skilled in the art that the compounds of formula (I) may be modified to provide pharmaceutically acceptable derivatives thereof at any of the functional groups in the compounds. Of particular interest as such derivatives are compounds modified at the C-1 carboxyl function, the C-7 or C-9 hydroxyl functions or at amino groups. Thus compounds of interest include C-1 alkyl (such as methyl, ethyl or propyl e.g. isopropyl) or aryl (e.g. phenyl, benzyl) esters of the compounds of formula (I), C-7 or C-9 esters of compounds of formula (I) such as acetyl esters thereof and C-7 or C-9 ethers such as phenyl ethers, benzyl ethers, and *p*-tolyl ethers and acylated amino derivatives such as formyl, acetamido.

It will be appreciated by those skilled in the art that the pharmaceutically acceptable derivatives of the compounds of formula (I) may be derivatised at more than one position.

Pharmaceutically acceptable salts of the compounds of formula (I) include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicyclic, succinic, toluene-*p*-sulphonic, tartaric, acetic, citric, methane-sulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other acids such as oxalic, while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining compounds of the invention and their pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium), ammonium and $(NR_4)^+$ (wherein R is C_{1-4}alkyl) salts.

References hereinafter to a compound f the invention include the compounds of formula (I) and pharmaceutically acceptable salts and derivatives thereof:

It will be appreciated by those skilled in the art that the nomenclature of the compounds of formula (I) can be defined in a number of ways. The compounds of formula (I) are, generically, substituted analogues of 2,6-dideoxy-2,3-didehydro-6-thio-N-acetylneuraminic acid; thus the following names are synonymous:-

5-Acetamido-2,6-anhydro-4-substituent-3,4,5,6-tetradeoxy-6-thio-D-*glycero*-D-*galacto*-non-2-enonic acid

5-Acetamido-4-*substituent*-2,3,4,5,6-pentadeoxy-6-thio-D-*glycero*-D-*galacto*-non-2-enopyranosonic acid.

The compounds of formula (I) possess anti-viral activity. In particular these compounds are inhibitors of viral neuraminidase in particular neuraminidase of orthomyxoviruses and paramyxoviruses, for example the viral neuraminidase of influenza A and B, parainfluenza, mumps and Newcastle disease.

There is thus provided in a further aspect of the invention a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof for use as an active therapeutic agent, in particular as an anti-viral agent, for example in the treatment of orthomyxovirus and paramyxovirus infections.

In a further or alternative aspect there is provided a method for the treatment of a viral infection, for example orthomyxovirus and paramyxovirus infections in a mammal, including man, comprising the step of administration of an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof.

There is also provided in a further or alternative aspect use of a compound of the invention for the manufacture of a medicament for the treatment of a viral infection.

It will be appreciated by those skilled in the art that reference herein to treatment extends to prophylaxis as well as to the treatment of established infections or symptoms.

It will be further appreciated that the amount of a compound of the invention required for use in treatment will vary not only with the particular compound selected, but also with the route of administration, the nature of the condition being treated, and the age and condition of the patient, and will ultimately be at the discretion of the attendant physician or veterinarian. In general, however, a suitable dose will be in the range of from about 0.1 to 750 mg/kg of body weight per day, preferably in the range of 0.5 to 60 mg/kg/day, most preferably in the range of 1 to 20 mg/kg/day.

Treatment is preferably commenced before or at the time of infection and continued until virus is no longer present in the respiratory tract. However the compounds are also effective when given post-infection, for example after the appearance of established symptoms.

Suitably treatment is given 1-4 times daily and continued for 3-7 days, e.g. 5 days post infection, depending upon the particular compound used.

The desired dose may be presented in a single dose or as divided doses administered at appropriate intervals, for example as two, three, four or more sub-doses per day.

The compound is conveniently administered in unit dosage form, for example containing 1 to 1500 mg, conveniently 3 to 700 mg, most conveniently 5 to 70 mg of active ingredient per unit dosage form.

While it is possible that for use in therapy a compound of the invention may be administered as the raw chemical, it is preferable to present the active ingredient as a pharmaceutical formulation.

The invention thus further provides a pharmaceutical formulation comprising a compound of the

formula (I) or a pharmaceutically acceptable salt or derivative thereof together with one or more pharmaceutically acceptable carriers therefor and optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not being deleterious to the recipient thereof. The pharmaceutical formulations may be in the form of conventional formulations for the intended mode of administration.

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration or in a form suitable for administration by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical formulations suitable for oral administration may conveniently be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution, a suspension or as an emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with

water or ther suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

The compound according to the invention may also be formulated for parenteral administration (e.g. by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The composition may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution, for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

For topical administration to the epidermis the compounds according to the invention may be formulated as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening agents, or colouring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising active ingredient in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most

preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the active compound with the softened or melted carrier(s) followed by chilling and shaping in moulds.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

For administration to the respiratory tract (including intranasal administration) according to the method of the invention, the neuraminidase inhibitors of general formula (I) may be administered by any of the methods and formulations employed in the art for intranasal administration, or administration by inhalation or insufflation via the mouth.

Thus in general the compounds may be administered in the form of a solution or a suspension or as a dry powder.

Solutions and suspensions will generally be aqueous for example prepared from water alone (for example sterile or pyrogen-free water) or water and a physiologically acceptable co-solvent (for example ethanol, propylene glycol, polyethylene glycols such as PEG 400).

Such solutions or suspensions may additionally contain other excipients, for example preservatives (such as benzalkonium chloride), solubilising agents/surfactants such as polysorbates (e.g. Tween 80, Span 80, benzalkonium chloride), buffering agents, isotonicity-adjusting agents (for example sodium chloride), absorption enhancers and viscosity enhancers. Suspensions may additionally contain suspending agents (for example microcrystalline cellulose, carboxymethyl cellulose sodium).

Solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a

dropper, pipette or spray. The formulations may be provided in single or multidose form. In the latter case a means of dose metering is desirably provided. In the case of a dropper or pipette this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray this may be achieved for example by means of a metering atomising spray pump.

Intranasal administration may also be achieved by means of an aerosol formulation in which the compound is provided in a pressurised pack with a suitable propellant such as a chlorofluorocarbon (CFC), for example dichlorodifluoromethane, trichlorofluoromethane or dichlorotetrafluoroethane, carbon dioxide or other suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by provision of a metered valve.

Solutions or suspensions are described above may also be administered to the respiratory tract via the mouth, for example, by means of a nebuliser.

Alternatively the compounds may be provided in the form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidine (PVP). For intranasal administration, conveniently the powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form for example in capsules or cartridges of e.g. gelatin or blister packs from which the powder may be administered by means of an inhaler.

In the formulations for administration to the respiratory tract, the compound will generally have a small particle size, for example of the order of 5 microns or less. Such a particle size may be obtained by means known in the art, for example by micronisation.

Preferably the formulation is suitable for intranasal administration, and may be presented as a liquid

spray or dispersible powder or in the form of drops.

Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilising agents or suspending agents. Liquid sprays are conveniently delivered from pressurised packs, which may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas.

When desired, formulations adapted to give sustained release of the active ingredient may be employed.

The compounds of the invention may also be used in combination with other therapeutic agents, for example other anti-infective agents. In particular the compounds of the invention may be employed with other anti-viral agents. The invention thus provides in a further aspect a combination comprising a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof together with another therapeutically active agent, in particular an anti-viral agent.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation, and thus such formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier therefor comprise a further aspect of the invention.

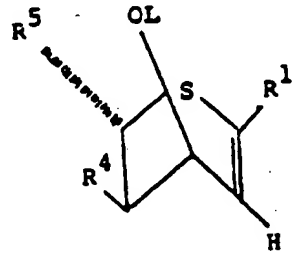
Suitable therapeutic agents for use in such combinations include other anti-infective agents, in particular anti-bacterial and anti-viral agents such as those used to treat respiratory infections. For example, other compounds effective against influenza viruses, such as amantadine, rimantadine and ribavirin, may be included in such combinations.

The individual components of such combination may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

When the c mp unds of the invention are used with a second therapeutic agent active against the same virus, the dose of each compound may either be the same as or differ from that employed when each compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

The compounds of formula (I) and their pharmaceutically acceptable salts and derivatives may be prepared by any method known in the art for the preparation of compounds of analogous structure. In particular the compounds of formula (I) may be prepared by the methods described below which form a further aspect of the invention.

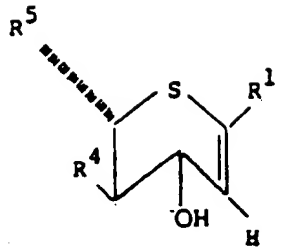
In one preferred embodiment, the invention provides a method for the preparation of a compound of formula (I), comprising the steps of subjecting a 2,3,5,6-tetradeoxy-4',5'-dihydro-2'-methyloxazolo[5,4-D-6-thio-D-*glycero*-β-D-*talo*-non-2-enopyranosonate to hydrolysis to give a compound of general formula (III),



(III)

wherein R^1, R^4 and R^5 are as defined in general formula (I), and OL is a leaving group such as a sulphonic acid residue, for example tosyl, mesyl, or trifluoromesyl, or a protected derivative of a compound of formula (III), and reacting the compound of formula (III) with an appropriate nucleophile, for example azide, cyanide, an appropriate carbanion, or thioacetate.

Compounds of formula (III) may be obtained from the corresponding compounds of formula (IV)



(IV)

by inversion of the 4-hydroxy group by methods known in the art, for example by reaction with a Lewis acid such as BF_3 etherate, followed by hydrolysis. Compounds of formula (IV) are either known in the art, or may be obtained by methods analogous to those for preparing the known compounds referred to herein, or methods analogous to those described in WO 91/16320.

Compounds of formula (I) may also be prepared from other compounds of formula (I) by interconversion. Thus compounds of formula (I) wherein R^3 is NH_2 or CH_2NH_2 may be prepared by reduction of the corresponding azido or cyano analogues respectively. Compounds wherein R^3 is NH alkyl or guanidino may be prepared by derivatisation of the corresponding compounds wherein R^3 NH_2.

Some of the intermediate compounds described herein are themselves novel, and these compounds, namely methyl 3-*S*-acetyl-2-azido-2-deoxy-3-thio-α-D-mannopyranoside, 1,4,6-tri-*O*-acetyl-3-*S*-acetyl-2-azido-2-deoxy-3-thio-α-D-mannopyranose, methyl 5-acetamido-5,6-dideoxy-6-thio-D-*glycero*-β-D-*galacto*-non-2-ulo-pyranosonate, methyl 7,8,9-tri-*O*-acetyl-2,3,5,6-tetradeoxy-4',5'-dihydro-2'-methyloxazolo[5,4-d]-6-thio-D-*glycero*-β-D-*talo*-non-2-enopyranosonate, methyl 5-acetamido-7,8,9-tri-*O*-acetyl-4-azido-2,3,4,5,6-pentadeoxy-6-thio-D-*glycero*-D-*galacto*-non-2-enopyranosonate, and 5-acetamido-4-ammonium-2,3,4,5,6-pentadeoxy-6-thio-D-*glycero* -D-*galacto*-non-2-enopyranosonate, are clearly to be understood to be within the scope of this invention.

As will be appreciated by those skilled in the art, it may be necessary or desirable at any stage in the processes described herein to protect one or more sensitive groups in the molecule to prevent undesirable side reactions; the protecting group may be removed at any convenient subsequent stage in the reaction sequence.

The protecting groups used in the preparation of compounds of formula (I) may be used in a conventional manner. See for example 'Protective Groups in Organic Chemistry', Ed. J.F.W. McOmie (Plenum Press, 1973) or 'Protective Groups in Organic Synthesis' by Theodora W. Greene (John Wiley and Sons, 1981).

Conventional amino protecting groups may include for example aralkyl groups, such as benzyl, diphenylmethyl or triphenylmethyl groups, and acyl groups such as *N*-benzyloxycarbonyl or t-butoxycarbonyl. Thus, compounds of general formula (I) wherein one or both of the groups R^3 and R^4 represent hydrogen may be prepared by deprotection of a corresponding protected compound.

Hydroxy groups may be protected, for example, by aralkyl groups, such as benzyl, diphenylmethyl or triphenylmethyl groups; acyl groups, such as acetyl; silicon protecting groups, such as trimethylsilyl groups; or as tetrahydropyran derivatives.

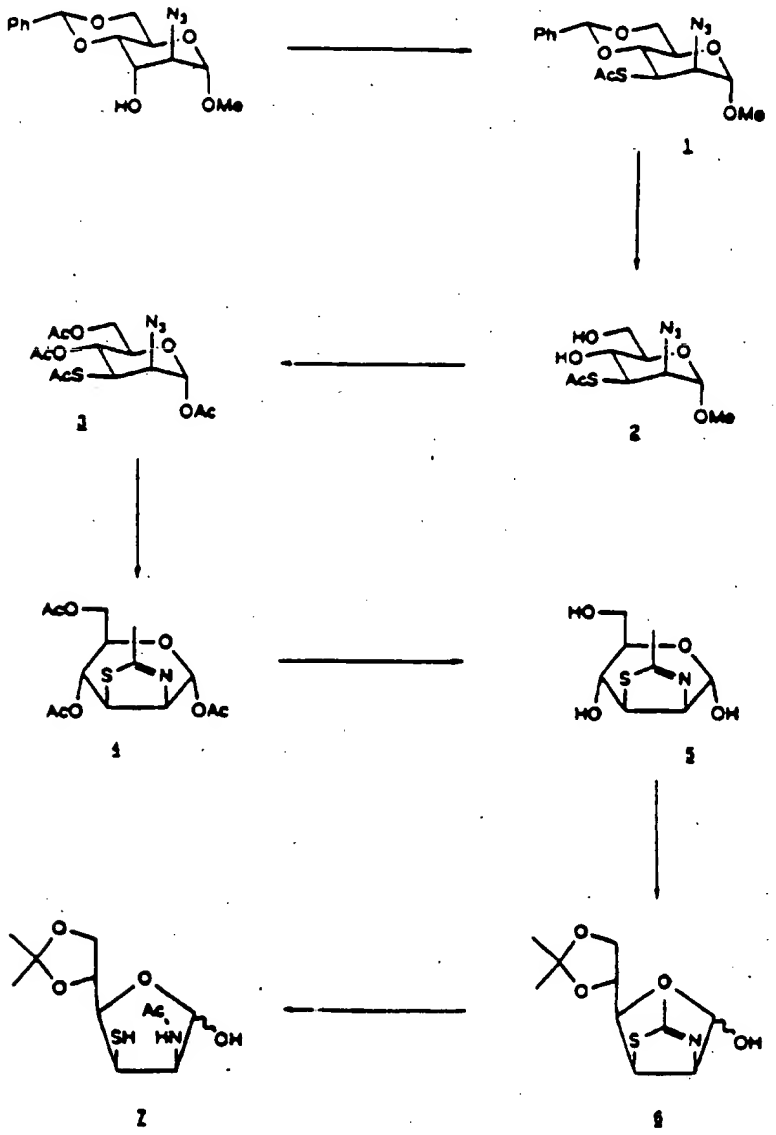
Removal of any protecting groups present may be achieved by conventional procedures. Thus an aralkyl group, such as benzyl, may be cleaved by hydrogenolysis in the presence of a catalyst, e.g. palladium on charcoal; an acyl group such as *N*-benzyloxycarbonyl may be removed by hydrolysis with, for example, hydrogen bromide in acetic acid, or by reduction, for example by catalytic hydrogenation; silicon protecting groups may be removed, for example, by treatment with fluoride ion; tetrahydropyran groups may be cleaved by hydrolysis under acidic conditions.

Where it is desired to isolate a compound of the invention as a salt, for example as an acid addition salt,

this may be achieved by treating the free base of general formula (I) with an appropriate acid, preferably with an equivalent amount, or with creatinine sulphate in a suitable solvent (e.g. aqueous ethanol).

The present invention is further described by the following examples which are for illustrative purposes only and should not be construed as a limitation of the invention.

Example 1 **Preparation of 2-Acetamido-2,3-dideoxy-5,6-O-isopropylidene-3-thio-D-mannofuranose (7)**



Preparation of methyl 3-*S*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-3-thio-α-D-mannopyranoside (1)

This compound was prepared following the literature method of Brossmer and Mack (Tetrahedron Letters, 1981 22 933) without modification.
^{13}C NMR ($CDCl_3$; δ(ppm) relative to TMS): 30.6, 44.8, 55.0, 64.2, 65.5, 68.7, 75.0, 98.9, 102.0, 126.0, 128.1, 128.9, 137.0, 193.8.

Preparation of methyl 3-*S*-acetyl-2-azido-2-deoxy-3-thio-α-D-mannopyranoside (2)

A mixture of the compound 1 (5.4 g; 14.7 mmol) and 50% aqueous acetic acid (60 mL) was heated at 100°C for 2 h, by which time the reaction mixture was homogeneous. The resulting yellow solution was cooled and concentrated. The residue was purified by putting it through a short plug of silica gel (the byproducts were removed by eluting the silica gel with diethyl ether/hexane (2:1); the desired product was eluted with ethyl acetate). Yield of the diol, obtained as a pale yellow solid, was 3.5 g (82%).

Preparation of 1,4,6-tri-O-acetyl-3-*S*-acetyl-2-azido-2-deoxy-3-thio-α-D-mannopyranose (3)

A solution of the diol 2 (11.25 g; 40.6 mmol) in acetic anhydride (120 mL) and acetic acid (120 mL) was chilled to 0°C prior to the addition of concentrated sulphuric acid (1 mL) dropwise over a 30 minute period. The ice bath was removed after 2 h and stirring was continued for 18 h. Sodium acetate (12 g) was added and after 15 minutes the reaction mixture was diluted with toluene (200 mL) and then evaporated to dryness. The residue was partitioned between diethyl ether (200 mL) and water (100 mL); the organic phase was washed with water (5 x 20mL), dried, and concentrated to give a pale yellow gum which solidified under high vacuum (13.5 g; 85%). A small portion of the product was recrystallized (ethyl acetate/hexanes) for analytical purposes; m.p. 87.5-88°.

^{1}H NMR ($CDCl_3$; δ(ppm) relative to TMS): 2.03, 2.09, 2.21 (s, 3x3H, $OCOCH_3$), 2.38 (SAc), 3.85 (d, *J* 1.3 Hz, 1H), 4.00-4.11 (m, 2H), 4.17-4.26 (m, 2H), 5.19 (dd, *J* 10.5, 10.5 Hz, 1H, H-4), 6.18 (s, 1H, H-1). ^{13}C NMR ($CDCl_3$; δ(ppm) relative to TMS): 20.5, 20.6, 20.9, 30.6, 45.1, 62.0, 62.9, 64.1, 71.9, 90.9, 168.4, 169.3, 170.6, 193.8. IR (KBr) ν_{max} cm^{-1} 2112 (m), 1750 (s), 1715 (m), 1370 (m), 1225 (s). $[\alpha]_D$ 5° (c, 1.07%, $CHCl_3$).

Preparation of 1,4,6-tri-O-acetyl-2-deoxy-2',3'-dihydro-2'-methylthiooxazolo-[2,3-d]-3-thio-α-D-mannopyranose (4)

A stirring solution of the azide 3 (13.5 g; 34.7 mmol) in dry dichloromethane (200 mL) was cooled to -78°C prior to the slow addition of a solution of triphenylphosphine (9.1 g; 34.7 mmol) in dry dichloromethane (200 mL) over a 2 h period. The reaction mixture was allowed to warm up to room temperature and stirring was continued for 18 h. The resulting solution was concentrated under vacuum before chromatographic separation (silica gel; eluting with ethyl acetate/hexane (1:3)) of the product from triphenylphosphine oxide. Colourless needles of the pure product were obtained by recrystallization from ethyl acetate/ hexane, 5.3 g (44%); m.p. 139 - 140°C (lit mp, Tetrahedron Letters, 1981 22 933, 135°C).

^{1}H NMR ($CDCl_3$; δ(ppm) relative to TMS): 2.00, 2.03, 2.10 (s, 3x3H, $OCOCH_3$); 2.24, (app. d, *J* 2Hz, 3H, N=C-CH_3); 3.75 (dd, 1H, $J_{3,2}$ 6.2 Hz, $J_{3,4}$ 10.0 Hz, H-3); 3.91 (ddd, 1H, $J_{5,4}$ 10.2 Hz, $J_{5,6}$ 5.3 Hz, $J_{5,6'}$ 2.9 Hz, H-5), 3.99 (dd, 1H, $J_{6,6'}$ 12.2 Hz, $J_{6,5}$ 5.3 Hz, H-6), 4.05 (dd, 1H, $J_{2,3}$ 6.2 Hz, $J_{2,1}$ 2 Hz, H-2), 4.10 (dd, 1H, $J_{6',6}$ 12.2 Hz, $J_{6',5}$ 2.9 Hz, H-6'), 4.80 (dd, 1H, $J_{4,3}$ 10.0 Hz, $J_{4,5}$ 10.2 Hz, H-4), 6.76 (br s, 1H, H-1); ^{13}C NMR ($CDCl_3$; δ(ppm) relative to TMS) 20.6, 20.77, 20.82, 52.0, 62.4 (C6), 68.7, 69.9, 77.9, 91.1 (C1), 168.3, 168.4, 169.4, 170.6. Anal. calculated for $C_{14}H_{19}NO_7S$: C,48.67; H, 5.54; N, 4.06. Found : C, 48.77; H, 5.64; N,

3.93. $[\alpha]_D$ -57° (c, 1.22%, $CHCl_3$).

Preparation of 2-deoxy-2',3'-dihydro-2'-methylthiooxazolo-[2,3-d]-3-thio-α,β-D-mannopyranose (5)

A mixture of the triacetate 4 (10 g; 29 mmol) and Amberlite IRA 400 (OH^-) (10 g) in methanol (250 mL) was stirred at room temperature for 48 h. The filtrate obtained after resin removal was concentrated to a solid (5.4 g; 86%); m.p. 185-189°C (lit mp, Tetrahedron Letters, 1981 22 933, 192-195°C). The triol (5) was used in the next step without further purification.

Alternatively, compound 4 can be deacetylated using a solution of sodium methoxide in methanol, in similar yield, following the literature method of Brossmer and Mack (Tetrahedron Letters, 1981 22 933).

^{13}C NMR (d_6-DMSO; δ(ppm) relative to TMS): 19.2, 56.2, 63.8, 72.0, 78.4, 89.6, 101.3, 165.9

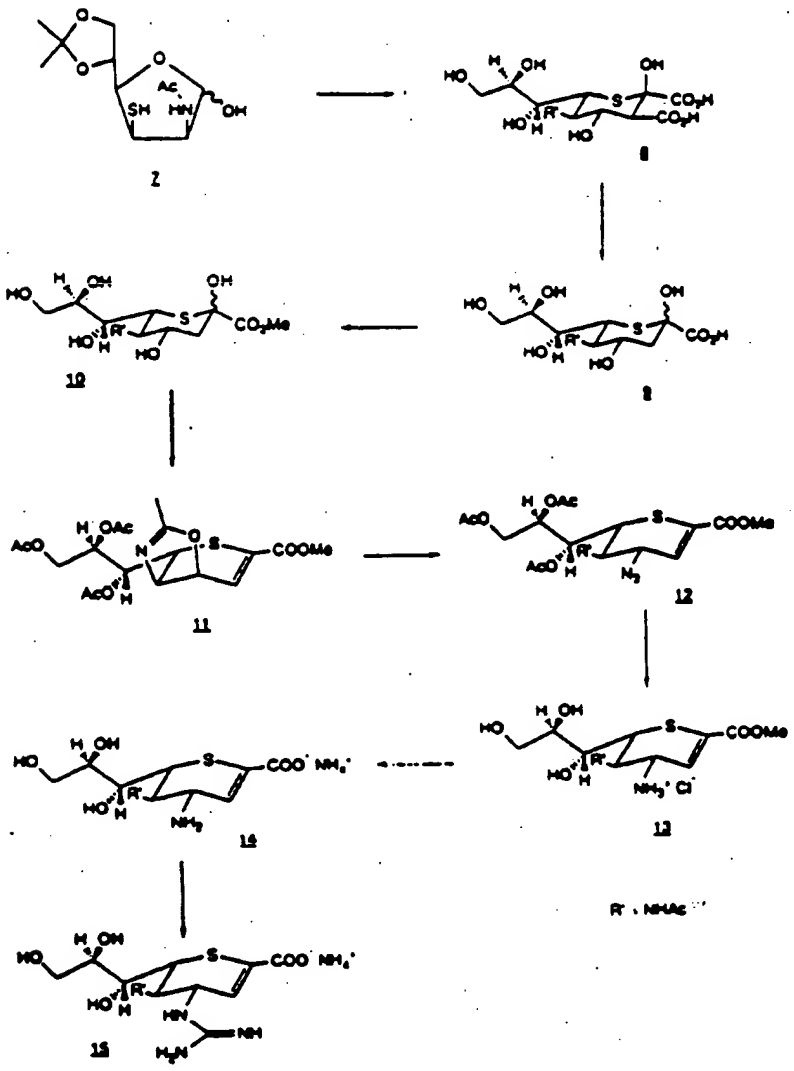
Preparation of 2-acetamido-2,3-dideoxy-5,6-O-isopropylidene-3-thio-D-mannofuranose (7)

This compound was prepared from the triol 5 following the literature method of Brossmer and Mack (Tetrahedron Letters, 1987 28 191).

^1H NMR of 2-deoxy-2',3'-dihydro-5,6-O-isopropylidene-2'-methylthiooxazolo-[2,3-d]-3-thio-β-D-mannofuranose (6) ($CDCl_3$; δ(ppm) relative to TMS): 1.35, 1.41 (s, 2x3H, $C(CH_3)_2$); 2.22 (s, 3H, N=C-CH_3); 3.96 (dd, 1H, $J_{6',6}$ 8.3 Hz, $J_{6',5}$ 4.6 Hz, H-6'); 4.12 (dd, 1H, $J_{6,6'}$ 8.3 Hz, $J_{6,5}$ 5.8 Hz, H-6), 4.24 (ddd, 1H, $J_{5,6}$ 5.8 Hz, $J_{5,6'}$ 4.6 Hz, $J_{5,4}$ 8.5 Hz, H-5), 4.38 (dd, 1H, $J_{4,5}$ 8.5 Hz, $J_{4,3}$ 5.6 Hz, H-4), 4.57 (dd, 1H, $J_{3,4}$ 5.6 Hz, $J_{3,2}$ 8.6 Hz, H-3), 5.12 (dd, 1H, $J_{2,3}$ 8.6 Hz, $J_{2,1}$ 1.5 Hz, H-2), 5.47 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1).

^1H NMR of 7 ($CDCl_3/CD_3OD$; δ(ppm) relative to TMS): 5.53 (s, 1H, H-1β); 5.28 (d, 1H, H-1α).

Example 2 Preparation of Ammonium 5-acetamido-2,3,4,5,6-pentadeoxy-4-guanidino-6-thio-D-glycero-β-D-galacto-non-2-enopyranosonate (15)



Preparation of 5-acetamido-5,6-dideoxy-6-thio-D-*glycero*-β-D-*galacto* -non-2-ulo-pyranosonic acid (9)

To an ice-cooled slurry of oxalacetic acid (3.75 g; 28.39 mmol) in water (13 mL) was added a solution of sodium hydroxide (2.27 g; 56.75 mmol) in water (12 mL) resulting in a solution of pH 7 - 7.5. The oxalacetate solution and nickel acetate tetrahydrate (0.94 g; 3.78 mmol) were added to the compound 7 (3.42 g; 9.46 mmol) and the mixture stirred at room temperature for 16 h. Amberlite IR-120 (H^+) resin was added to pH 3. After stirring for six h, the resin was filtered off and the filtrate was lyophilised. The residue was purified by column chromatography (Amberlite IRA-400 ($HCOO^-$ form) eluting with formic acid (2N)) affording the diacid 8 (2.38 g; 68%) (Brossmer and Mack, Tetrahedron Letters, (1987) 28 191 and H. Hagedorn et al, Carbohydrate Research, 1992 236 89).

^1H NMR (D_2O; δ(ppm) relative to HOD = 4.7): 2.04 (s, 3H, Ac); 3.36 (d, 1H, $J_{3,4}$ 10.2 Hz, H-3); 3.47 - 3.57 (m, 3H, H-6, H-8, H-9'); 3.76 (m, 1H, H-9); 3.91 (m, 1H, H-7); 4.10 (pseudo triplet, 1H, $J_{4,5}$ 9.9 Hz, H-4); 4.18 (pseudo triplet, 1H, $J_{5,6}$ 10.5 Hz, $J_{5,NH}$ 10.5 Hz, H-5). ^{13}C NMR (D_2O; δ(ppm) relative to TMS): 25.8 ($NHCOCH_3$); 48.0 (C6); 59.1 (C-5); 62.3 (C-3); 66.7 (C-9); 72.0 (C-8); 73.7 (C-4); 74.6 (C-7); 85.5 (C-2); 176.3, 177.2, 178.8 (carbonyls).

A solution of the diacid 8 (2.38 g; 6.45 mmol) in water (200 mL) was adjusted to pH 6 with 0.1M Na_2HPO_4 solution and heated at 90°C for 5 - 6 h. The resulting solution was then purified on Amberlite IRA-400 ($HCOO^-$ form) eluting successively with water (1 litre) and HCOOH (2N). The latter fraction was lyophilised, affording compound 9 as a solid (1.62 g; 77%).

^1H NMR (D_2O; δ(ppm) relative to HDO = 4.7): 2.03(s, 3H, $NHCOCH_3$); 2.24 (dd, 1H, $J_{3a,3e}$ 11.2 Hz, $J_{3a,4}$13.3 Hz, H-3a);

2.46 (dd, 1H, $J_{3e,4}$ 4.4 Hz, H-3e); 3.42 (dd, 1H, $J_{6,5}$ 10.1 Hz, $J_{6,7}$ 1.6 Hz, H-6); 3.54 (dd, 1H, $J_{9',8}$ 5.8 Hz, $J_{9',9}$ 10.1 Hz, H-9'); 3.60 (ddd, 1H, $J_{8,7}$ 9.2 Hz, $J_{8,9}$ 5.1 Hz, H-8); 3.75 (dd, 1H, H-9); 3.83 (dd, 1H, H-7); 3.92 (ddd, 1H, $J_{4,5}$ 10.7 Hz, H-4); 4.03 (pseudo triplet, 1H, H-5). ^{13}C NMR (D_2O; δ(ppm) relative to TMS): 24.6 ($NHCOCH_3$); 46.9 (C-3); 47.2 (C-6); 58.4 (C-5); 63.2 (C-9); 70.9, 71.3 (C-4, C-7); 73.4 (C-8); 83.8 (C-2); 177.6 (carbonyls).

Preparation of methyl 5-Acetamido-5,6-dideoxy-6-thio-D-glycero-β-D-galacto-non-2-ulo-pyranosonate (10)

Dowex 50W-X8 (H^+) (2 mL) was added to a solution of the acid 9 (2.16 g; 6.65 mmol) in dry methanol (200 mL) and the mixture was stirred at room temperature for 48 h. The resin was filtered off and the filtrate concentrated giving the ester 10 as a solid (1.91 g; 85%).

^{1}H NMR (D_2O; δ(ppm) relative to HOD = 4.7): 2.03 (s, 3H, $NHCOCH_3$); 2.24 (dd, 1H, $J_{3a,3e}$ 13.1 Hz, $J_{3a,4}$ 11.3 Hz, H-3a); 2.49 (dd, 1H, $J_{3e,4}$ 4.4 Hz, H-3e); 3.50 - 3.59; 3.70 - 3.85 (m, 8H, H-5, H-6, H-8, H-9, H-9', $COOCH_3$); 3.92 (ddd, 1H, $J_{4,5}$ 10.8 Hz, H-4); 4.02 (pseudo triplet, 1H, $J_{5,6}$ 10.2 Hz, H-5). ^{13}C NMR (D_2O; δ(ppm) relative to TMS): 24.7 ($NHCOCH_3$), 46.5 (C-3), 47.3 (C-6), 56.5, 58.4 (C-5, $COOCH_3$), 65.7 (C-9), 71.0, 71.1, 73.5 (C-4, C-7, C-8) 83.2 (C-2), 175.4, 177.7 (carbonyls). MS (FAB): 340 $(M+1)^+$. MS (High Resolution FAB for $C_{12}H_{22}NO_8S$): 340.1066 (calculated); 340.1075 (observed).

Preparation of methyl 7,8,9-tri-O-acetyl-2,3,5,6-tetradeoxy-4',5'-dihydro-2'-methyloxazolo[5,4-d]-6-thio-D-glycero-β-D-talo-non-2-enopyranosonate (11)

Concentrated sulphuric acid (0.4 mL) was added to a solution of the ester 10 (0.973 g; 2.87 mmol) in acetic anhydride (4 mL) and glacial acetic acid (4 mL), and the resulting solution stirred at room temperature for 40 h. The solution was poured into a stirred saturated solution

of sodium bicarbonate, stirred f r 1 h bef re extraction with ethyl acetate. The extracts were dried (Na_2SO_4) and concentrated to afford the oxazoline 11 as a syrup (1.2g; 97%); 95% pure, by ^1H NMR. This was used in the subsequent step without further purification.

^1H NMR ($CDCl_3$; δ(ppm) relative to TMS): 2.04, 2.09, 2.11, 2.15 (s, 4 x 3H, $OCOCH_3$, oxazoline CH_3); 2.75 (dd, 1H, $J_{6,5}$ 10.7 Hz, $J_{6,7}$ 2.9 Hz, H-6); 3.84 (s, 3H, $COOCH_3$); 4.14 (dd, 1H, $J_{9,8}$ 6.3 Hz, $J_{9,9'}$ 12.2 Hz, H-9); 4.25 (dd, 1H, $J_{5,4}$ 9.4 Hz, H-5); 4.37 (dd, 1H, $J_{9',8}$ 3.2 Hz, H-9'); 4.89 (dd, 1H, $J_{4,3}$ 3.5 Hz, H-4); 5.42 (ddd, 1H, $J_{8,7}$ 5.9 Hz, H-8); 5.77 (dd, 1H, H-7); 7.20 (d, H-3). ^{13}C NMR ($CDCl_3$; δ(ppm) relative to TMS): 14.0, 20.5, 20.6, 20.8 (s, 4 x 3H, =C-CH_3, $COCH_3$); 47.0 (C-6); 52.8 ($COOCH_3$); 61.7 (C-9); 67.5 (C-5); 69.2 (C-7); 70.8 (C-8); 75.8 (C-4); 129.9 (C-3); 131.8 (C-2); 163.8, 165.9, 169.5, 169.8, 170.5 (N=C-, carbonyls). MS (FAB): 430 $(M+1)^+$. MS (High Resolution FAB for $C_{18}H_{24}NO_9S$): 430.1172 (calculated); 430.1189 (observed). $[\alpha]_D$-47° (c, 1.37%, $CHCl_3$).

<u>Preparation of methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5,6-pentadeoxy-6-thio-D-glycero-D-galacto-non-2-enopyranosonate</u> (12)

The oxazoline 11 (1.04 g; 2.42 mmol) was treated with azidotrimethylsilane (0.96 mL; 7.26 mmol) in 2-methyl-2-propanol (10 mL) at 75 - 80°C for 48 h. The solution was cooled, added to saturated sodium bicarbonate, and after 1 h, extracted with ethyl acetate (3 x 50 mL). The extracts were dried (Na_2SO_4) and solvent removal gave a syrup (1.01 g). This was purified by column chromatography on silica gel, by elution with ethyl acetate / hexane (3:1), which afforded the azide 12 as a foam (0.75g; 66%).

^1H NMR ($CDCl_3$; δ(ppm) relative to TMS): 2.04, 2.06, 2.10, 2.11 (s, 4 x 3H, $NHCOCH_3$, $COCH_3$); 3.82 (s, 3H, $COOCH_3$); 3.85 (dd, 1H, $J_{6,5}$ 6.4 Hz, $J_{6,7}$ 4.8 Hz, H-6); 4.01 (ddd, 1H,

$J_{5,4}$ 6.9 Hz, $J_{5,NH}$ 7.9 Hz, H-5); 4.16 (dd, 1H, $J_{9,9',8}$ 5.8 Hz, $J_{9,9'}$ 12.2 Hz, H-9'); 4.36 (dd, 1H, $J_{9,8}$ 3.7 Hz, H-9); 4.67 (dd, 1H, $J_{4,3}$ 3.9 Hz, H-4); 5.37 (ddd, 1H, $J_{8,7}$ 6.1 Hz, H-8); 5.49 (dd, 1H, H-7); 6.01 (d, 1H, NH); 6.83 (d, 1H, H-3). ^{13}C NMR ($CDCl_3$; d(ppm) relative to TMS): 20.5, 20.7, 23.1 ($OCOCH_3$, $NHCOCH_3$); 43.4 (C-6); 46.8 (C-5); 52.9 ($COOCH_3$); 59.0 (C-4); 61.5 (C-9); 68.2 (C-7); 69.8 (C-8); 127.7 (C-3); 129.1 (C-2); 163.4, 169.6, 169.9, 170.5, 170.6 (carbonyls). IR (NaCl) ν_{max} (cm^{-1}): 2104 ($-N_3$). MS (FAB): 473 $(M+1)^+$, 430 (M^+-N_3). MS (High Resolution FAB for $C_{18}H_{23}N_4O_9S$): 473.1342 (calculated); 473.1339 (observed). $[\alpha]_D$ 73° (c, 0.31%, $CHCl_3$).

Preparation of ammonium 5-acetamido-4-amino-2,3,4,5,6-pentadeoxy-6-thio-D-glycero-D-galacto-non-2-enopyranosonate (14)

A mixture of the azide 12 (126 mg; 0.267 mmol), zinc dust (69.7 mg; 1.067 mmol) and 2N HCl (2 mL) was heated at 75°C for 45 min. The resulting solution was concentrated to dryness yielding the amine hydrochloride, 5-acetamido-4-ammonium-2,3,4,5,6-pentadeoxy-6-thio-D-*glycero* -D-galacto-non-2-enopyranosonate (13), as a straw coloured foam (140 mg).

To the foam in water (5 mL) was added sodium hydroxide to pH 13 and the solution stirred at room temperature for 16 h. The pH was adjusted to 7.5 using Dowex 50W-X400 (H^+). The resin was filtered off and the filtrate purified on a Dowex 50W-X400 (H^+) column. The column was washed with water (200 mL), and then eluted with 1.5N NH_4OH solution; the latter fraction afforded the amine 14 (57 mg; 66%) as a foam after lyophilisation.

^1H NMR (D_2O; δ(ppm) relative to HOD = 4.7): 2.00 (s, 3 H, $NHCOCH_3$), 3.52 (dd, 1 H, $J_{9,9',8}$ 5.8 Hz, $J_{9,9'}$ 11.4 Hz, H-9'); 3.63 (m, 1 H, H-8); 3.68-3.83 (m, 3 H, H-4, H-6, H-9), 4.08 (pseudo doublet, $J_{7,8}$ 8.8 Hz, H-7), 4.29 (pseudo triplet, 1 H, $J_{5,4}$ 9.5 Hz, $J_{5,6}$ 9.5 Hz, H-5); 6.34 (br s, 1 H, H-3).

^{13}C NMR (D_2O; external reference $CHCl_3$): 24.8 ($NHCOCH_3$); 47.1 (C-6); 51.5 (C-5); 54.7 (C-4); 65.5 (C-9); 70.9 (C-8); 73.5 (C-7); 124.0 (C-3); 139.1 (C-2); 172.4; 177.4 (C-1, $NHCOCH_3$) * tentative assignments

A small amount of the amine 14 was desalted (HPLC; Waters Millipore μBondapak C18 reverse phase; eluant: 0.1% TFA in H_2O) to give the corresponding acid, for microanalytical and NMR characterisation purposes. ^1H NMR (D_2O; δ(ppm) relative to HOD = 4.7): 2.09 (s, 3 H, $NHCOCH_3$); 3.62 (dd, 1 H, $J_{9',8}$ 5.6 Hz, $J_{9',9}$ = 11.6 Hz, H-9'); 3.72 (m, 1 H, H-8); 3.82 (pseudo doublet, 1 H, H-9); 3.86, 3.94 (pseudo doublet x 2, 1 H x 2, J 9.8 Hz and 9.5 Hz, respectively, H-4, H-6); 4.29 (dd, 1 H, $J_{7,6}$ 2.5 Hz, $J_{7,8}$ 9.4 Hz, H-7); 4.45 (pseudo triplet, 1 H, $J_{5,4}$ 10.0 Hz, $J_{5,6}$ 10.0 Hz, H-5); 6.61 (br s, 1 H, H-3).

MS (FAB): 307 $(M+1)^+$, 290 $(M^+ - NH_3)$. MS (High Resolution FAB for $C_{11}H_{19}N_2O_6S$): 307.0964 (calculated); 307.0974 (observed). Anal. calculated for $C_{11}H_{18}N_2O_6S$: C, 27.59; H, 4.03; N, 4.17. Found: C, 27.52; H, 4.06; N, 4.20. $[\alpha]_D$ 18° (c, 1.42%, H_2O)

The amine hydrochloride 13 was characterised as its peracetate. Thus, the azide 12 (47.5 mg; 0.10 mmol) was treated with zinc dust and 2N HCl as described above. The crude amine hydrochloride 13 was then treated with acetic anhydride (1 mL) and concentrated sulphuric acid (0.1 mL) at room temperature for 16 h before pouring into saturated sodium bicarbonate solution. After extraction with acetonitrile (5 x 10 mL) and solvent removal, an oil (39 mg) was obtained. This was chromatographed (silica gel; eluting with ethyl acetate/methanol (19:1)), affording methyl 5,6-diacetamido-7,8,9-tri-*O*-acetyl-2,3,4,5,6-pentadeoxy-6-thio-D-*glycero*-β-D-*galacto*-non-2-enopyranosonate (25 mg; 60%) as a syrup.

^{1}H NMR ($CDCl_3$; δ(ppm) relative to TMS): 1.95; 2.00 2.06, 2.07, 2.11 (s, 5 x 3H, Ac); 3.66 (dd, 1H, $J_{6,5}$ 8.9 Hz, $J_{6,7}$ 4.6 Hz, H-6); 3.80 (s, 3H, $COOCH_3$); 4.15 (dd, 1H, $J_{9',8}$ 5.8 Hz, $J_{9',9}$ 12.4 Hz, H-9'); 4.38 (dd, 1H, $J_{9,8}$ 3.5 Hz, H-9); 4.38 (m, 1H, H-5); 4.85 (ddd, 1H, $J_{4,3}$ 3.0 Hz, $J_{4,5}$ 9.1 Hz, $J_{4,NH}$ 9.1 Hz, H-4); 5.21 (ddd, 1H, $J_{8,7}$ 6.3 Hz, H-8); 5.52 (dd, 1H, H-7); 6.16; 6.41 (d, 2 x 1H, NH); 6.70 (d, 1H, H-3). ^{13}C NMR ($CDCl_3$; d(ppm) relative to TMS): 20.5, 20.7, 20.9, 23.0 ($OCOCH_3$, $NHCOCH_3$); 44.3 (C-6); 49.3, 50.4 (C-4, C-5); 52.9 ($COOCH_3$); 61.6 (C-9); 67.6, 70.3 (C-7, C-8); 128.1 (C-2); 131.1 (C-3); 163.6, 169.9, 170.6, 171.0 (carbonyls). MS (FAB): 489 (M+1)$^+$. MS (High Resolution FAB for $C_{20}H_{29}N_2O_{10}S$): 489.1543 (calculated); 489.1542 (observed). $[\alpha]_D$ -13° (c, 0.40%, $CHCl_3$).

Preparation of ammonium 5-acetamido-2,3,4,5,6-pentadeoxy-4-guanidino-6-thio-D-*glycero*-β-D-*galacto*-non-2-enopyranosonate (15)

To a solution of the amine 14 (40.0 mg; 0.124 mmol) and imidazole (33.8 mg; 0.496 mmol) in water (1mL) was added pyrazole carboxamidine hydrochloride (33.4 mg; 0.248 mmol). The solution was stirred at 60°C for 24 h and then chromatographed (silica gel; eluting with 80% isopropanol) to provide compound 15 (25 mg; 55%).

^{1}H NMR (D_2O; δ(ppm) relative to HDO = 4.7): 1.96 (s, 3 H, $NHCOCH_3$), 3.53 (dd, 1 H, $J_{9',8}$ 5.7 Hz, $J_{9',9}$ 11.6 Hz, H-9'); 3.63 (m, 1 H, H-8), 3.73-3.86 (m, 3 H, H-4, H-6, H-9), 4.22 (pseudo triplet, 1 H, $J_{5,4}$ 9.3 Hz, $J_{5,6}$ 9.3 Hz, H-5), 4.40 (pseudo doublet, 1 H, $J_{7,8}$ 8.6 Hz, H-7), 6.32 (br s, 1 H, H-3).

^{13}C NMR (D_2O; external reference $CHCl_3$): 24.7 ($NHCOCH_3$); 47.1 (C-6); 53.2 (C-5); 56.2 (C-5); 65.8 (C-9); 71.4 (C-7); 73.8 (C-8); 130.0 (C-3); 137.6 (C-2); 159.7 (C-10 guanidinium group); 173.1; 177.4 (C-1, $NHCOCH_3$) * tentative assignments

A small sample of the guanidium compound 15 was desalted (HPLC; Waters Millipore μB ndapak C18 reverse phase; eluant: 0.1% TFA in H_2O) to give the corresponding acid of 15. ^1H NMR (D_2O; δ(ppm) relative to HDO = 4.7): 1.98 (s, 3 H, $NHCOCH_3$); 3.59 (dd, 1 H, $J_{9,8}$ 5.0 Hz, $J_{9',9}$ 11.3 Hz, H-9'); 3.68 (m, 1 H, H-8); 3.81 (dd, 1 H, $J_{9,8}$ 1.1 Hz, H-9); 3.84, 3.92 (pseudo doublet x 2, 1 H x 2, J 9.2 Hz, 9.2 Hz, H-4, H-6), 4.30 (pseudo triplet, 1 H, $J_{5,4}$ 9.2 Hz, $J_{5,6}$ 9.2 Hz, H-5); 4.48 (dd, 1 H, $J_{7,6}$ 1.9 Hz, $J_{7,8}$ 8.8 Hz, H-7); 6.54 (br s, 1 H, H-3). MS (FAB): 349 $(M+1)^+$. MS (High Resolution FAB for $C_{12}H_{21}N_4O_8S$): 349.1182 (calculated); 349.1164 (observed).

Example 3 Biological Evaluation

Sialidase activity was assayed using the fluorimetric assay of Potier et al (Anal. Biochem., (1979), 94 287), as modified by Chong et al (Biochim. Biophys. Acta, 1991 1077 65). The concentration of the substrate, 4-methylumbelliferyl Neu5Ac, was 80mM.

	IC_{50} (M)
Compound 14	1×10^{-6}
Compound 15*	5×10^{-9}

* This compound was found to be a slow binding inhibitor.

Example 4

The following formulations are representative of compositions according to the invention:

Aqueous Solution	% w/w
Compound of formula (I)	10.0
Benzalkonium chloride	0.04
Phenylethyl alcohol	0.40
Purified water	to 100% w/w

Aqueous Cosolvent Solution	% w/w
Compound of formula (I)	10.0
Benzalkonium chloride	0.04
Polyethylene glycol 400	10.0
Propylene glycol	30.0
Purified water	to 100% w/w

Aerosol Formulation	%w/w
Compound of formula (I)	7.5
Lecithin	0.4
Propellant 11	25.6
Propellant 12	66.5

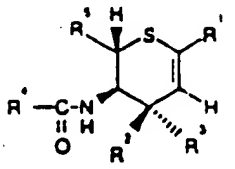
Dry Powder Formulation	% w/w
Compound of formula (I)	40.0
Lactose	60.0

These formulations are prepared by admixture of the active ingredient and excipients by conventional pharmaceutical methods.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

CLAIMS

1. A compound of formula (I)



(I)

wherein R^1 is COOH, $P(O)(OH)_2$, NO_2, SOOH, SO_3H, tetrazol, CH_2CHO, CHO, or $CH(CHO)_2$;

one of R^2 and R^3 is OH, $(alk)_xNR^6R^7$, CN or N_3, and the other is hydrogen, where alk is unsubstituted or substituted methylene, and x is 0 or 1, with the proviso that when R^2 or R^3 is OH R^1 cannot be COOH;

R^4 is methyl, in which one or more hydrogens is optionally replaced by a substituted or unsubstituted C_{1-4}alkyl or aryl group, or by a halogen;

R^5 is $CHOR^9CHOR^9CH_2OR^9$;

R^6 is hydrogen, C_{1-6}alkyl, allyl, aryl, aralkyl, amidine, NR^7R^8, or an unsaturated or saturated ring containing one or more heteroatoms;

R^7 is hydrogen, C_{1-6}alkyl, or allyl, or NR^6R^7 forms an optionally substituted 5 or 6 membered ring optionally containing one or more additional heteroatoms, or R^6 and R^7 may be the same; and

R^8 is hydrogen or C_{1-6}alkyl; and

each R^9 is the same or different, and is hydrogen; an acyl group having 1 to 4 carbon atoms; a linear or cyclic alkyl group having 1 to 6 carbon atoms, or a halogen-substituted analogue thereof; an allyl group or an unsubstituted aryl group; or an aryl substituted by a halogen, an OH group, an NO_2 group, an NH_2 group or a COOH group;

and pharmaceutically acceptable salts of the compounds of formula (I) and pharmaceutically acceptable derivatives thereof.

2. A compound according to Claim 1, wherein R^1 is COOH and R^2 is H.

3. A compound according to Claim 1 or Claim 2, wherein R^3 is NR^6R^7,

4. A compound according to Claim 3, wherein R^3 is NH_2 or guanidino.

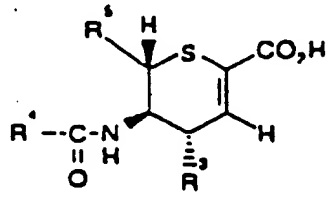
5. A compound according to any one of Claims 1 to 4, wherein R^4 is methyl or halogen-substituted methyl.

6. A compound according to any one of Claims 1 to 5, wherein R^4 is methyl.

7. A compound according to any one of Claims 1 to 5, wherein R^4 is FCH_2, F_2CH or CF_3.

8. A compound according to any one of Claims 1 to 7, wherein R^5 is H or acetyl.

9. A compound of formula (Ia)



(Ia)

wherein R^3 is hydrogen, OH, $(alk)_xNR^6R^7$, CN or N_3, where alk is unsubstituted or substituted methylene, and x is 0 or 1;

R^4 is methyl, in which one or more hydrogens is optionally replaced by a substituted or unsubstituted C_{1-6}alkyl or aryl group, or by a halogen;

R^5 is $CHOR^9CHOR^9CH_2OR^9$;

R^6 is hydrogen, C_{1-6}alkyl, allyl, aryl, aralkyl, amidine, NR^7R^8, or an unsaturated or saturated ring containing one or more heteroatoms;

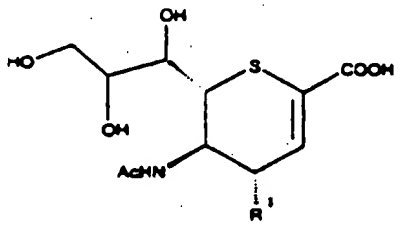
R^7 is hydrogen, C_{1-6}alkyl, or allyl, or NR^6R^7 forms an optionally substituted 5 or 6 membered ring optionally containing one or more additional heteroatoms, or R^6 and R^7 may be the same; and

R^4 is hydrogen or C_{1-6}alkyl; and

each R^3 is the same or different, and is hydrogen; an acyl group having 1 to 4 carbon atoms; a linear or cyclic alkyl group having 1 to 6 carbon atoms, or a halogen-substituted analogue thereof; an allyl group or an unsubstituted aryl group; or an aryl substituted by a halogen, an OH group, an NO_2 group, an NH_2 group or a COOH group;

and pharmaceutically acceptable salts and derivatives thereof.

10. A compound of formula II



(II)

wherein R^1 is NH_2 or $NHC(NH)(NH_2)$,

and physiologically acceptable derivatives and solvates thereof.

11. 5-Acetamido-2,3,4,5,6-pentadeoxy-4-guanidino-6-thio-D-glycero-β-D-galacto-non-2-enopyranosonate or a pharmaceutically-acceptable salt thereof.

12. Ammonium 5-acetamido-2,3,4,5,6-pentadeoxy-4-guanidino-6-thio-D-glycero-β-D-galacto-non-2-enopyranosonate.

13. 5-Acetamido-4-amino-2,3,4,5,6-pentadeoxy-6-thio-D-glycero-D-galacto-non-2-enopyranosonate.

14. Ammonium 5-Acetamido-4-amino-2,3,4,5,6-pentadeoxy-6-thio-D-glycero-D-galacto-non-2-enopyranosonate.

15. 5-Acetamido-4-azido-2,3,4,5,6-pentadeoxy-6-thio-D-glycero-D-galacto-non-2-enopyranosonate.

16. Methyl 5-Acetamido-4-azido-2,3,4,5,6-pentadeoxy-6-thio-D-glycero-D-galacto-non-2-enopyranosonate.

17. A compound according t any one of Claims 1 to 16 for use in medicine.

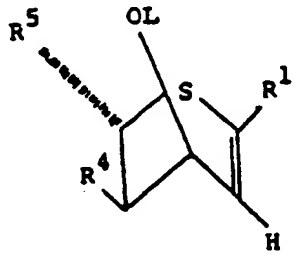
18. A compound according to any one of Claims 1 to 16, for use as an active therapeutic agent in the treatment of viral infections selected from orthomyxovirus infection or paramyxovirus infection.

19. Use of a compound according to any one of Claims 1 to 16 for the manufacture of a medicament for the treatment of a viral infection.

20. Use according to Claim 19 in which the viral infection is an orthomyxovirus infection or a paramyxovirus infection.

21. Use according to Claim 19 or Claim 20 in which the viral infection is influenza.

22. A method for the preparation of a compound of formula (I) as defined in claim 1, which comprises the steps of subjecting a 2,3,5,6-tetradeoxy-4',5'-dihydro-2'-methyloxazolo[5,4-D-6-thio-D-*glycero*-β-D-*talo*-non-2-enopyranosonate to hydrolysis to give a compound of general formula (III),



wherein R^1, R^4 and R^5 are as defined in general formula (I), and OL is a leaving group and reacting the compound of formula (III) with an appropriate nucleophile.

23. A method according to Claim 33 wherein methyl 7,8,9-tri-*O*-acetyl-2,3,5,6-tetradeoxy-4',5'-dihydro-2'-methyloxazolo [5,4-d]-6-thio-D-*glycero*-β-D-*talo*-non-2-enopyranosonate is hydrolysed.

24. A pharmaceutical formulation comprising a compound according to any one of Claims 1 to 16, together with a pharmaceutically-acceptable carrier.

25. A formulation according to Claim 24 adapted for administration to the respiratory tract.

26. A formulation according to Claim 24 adapted for intranasal administration.

27. A method for the treatment of viral infection in an animal comprising the step of administration of an effective amount of a compound according to any one of Claims 1 to 16 to an animal in need of such treatment.

28. A method according to Claim 27, wherein the compound is as defined in any one of Claims 9 to 16.

29. A method according to Claim 28, wherein the compound is as defined in any one of Claims 10 to 16.

30. A method according to any one of Claims 27 to 29, wherein the viral infection is infection by an orthomyxovirus or a paramyxovirus.

31. A method according to Claim 30, wherein the viral infection is influenza.

32. A method according to Claim 30 or Claim 31, wherein the animal is a human.

33. A method according to any one of Claims 27 to 32 wherein the compound is administered to the respiratory tract.

34. A method according to any one of Claims 27 to 32 wherein the compound is administered intranasally.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 95/00470

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C07D 335/02, A61K 31/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC C07D 335/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU; IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS-ONLINE SUBSTRUCTURE SEARCH
DERWENT, JAPIO IPC as above

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	AU,B, 77590/91 (654815) (BIOTA SCIENTIFIC MANAGEMENT PTY LTD), 31 October 1991 See whole document	1-34
Y	Tetrahedron Letters, Vol. 28, No. 2, pp 191-194, 1987, "Synthesis of 6- Thiosialic Acids and 6- Thio- N-acetyl -D- neuraminic acid", H.Mack and R.Brossner. See whole document.	1-34

☐ Further documents are listed in the continuation of Box C ☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 November 1995	15 NOVEMBER 1995

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application N .
PCT/AU 95/00470

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
AU	9177590	CN	1057260	CS	9101145	EP	526543
		FI	924790	HU	9203180		

END OF ANNEX